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Prostate Cancer is the most common cancer among African American males. Recent reports suggest that not only is the incidence higher than their white counterparts, but that they present at an earlier age with more diffuse disease, and have a higher morbidity and mortality. Our current study is designed to evaluate potential differences in tumor suppressor genes between cancers of non-Hispanic Whites and African Americans. We have selected the tumor suppressor genes BCL-2, p53 and Rb. We have selected cancers from America as well as South Africa to assess the potential for an increased virulence in African Americans compared to whites and Native Africans.

In this initial grant we are funded to establish a laboratory and to prepare to write a Department of Defense grant that was submitted in the spring of 1999.

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FOREWORD

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W Bah 7/29/99

PI - Signature Date

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INTRODUCTION

Prostate Cancer is the most common cancer among African American men. They appear to present with more diffuse premalignancy, at higher stage, and share an inordinate morbidity and mortality from this disease compared to their Caucasian counterparts. The incidence of prostate cancer in African Americans is almost twice that of non-Hispanic White Americans. The incidence of prostate cancer in African blacks is poorly understood. There is evidence that the incidence of blacks in Nigeria is closer to that of whites in America than that of American Blacks. There is also evidence in the Asian community that as Asians migrate to the United States, their incidence of Prostate cancer markedly increases compared to their counterparts on the Asian continent. The purpose of this grant is to begin an investigation into potential differences between prostate cancers from blacks and whites on the African continent and in the United States. We have chosen to look at specific differences of tumor proliferation and apoptosis, as potential markers of these differences. The markers to be evaluated in this round of funding are Bcl-2, p53 and Rb. The scope of research in this grant includes the establishment of an academic research presence, increase knowledge of the subject, demonstrate competency and prepare a New Investigator grant that was submitted In May of 1999.

BODY: Tasks

Phase 1: Project Startup

- **Meet with collaborating investigator and mentor:** I joined the faculty at UC Davis in October of 1998. My collaborating investigator and mentor Dr. R.W. deVere White and I first began to meet in the summer of 1998. We meet privately for 1 hour every two weeks, to discuss my progress and overall program. He provided me with bench space and an office in the lab
- **Open communication with Simon Reif**: Dr Simon Reif is the Chief of at the University of Pretoria, South Africa. I began to communicate with him in late 1998. We communicated first by phone and subsequently by E-mail. We have communicated monthly over a variety of topics including ways to increase the sample size by including the University of Orange Free State and Stellenbosch in South Africa. I have subsequent to these conversations requested Dr. Reif handle these negotiations and act as our South African Counterpart. These negotiations for further specimens are ongoing.
- **Purchase a computer**: the laboratory provided An IBM Pentium II computer. We used the funds for this grant to purchase a laptop, which is invaluable in allowing a mobile workplace. Currently I have a lab office at the University and the Northern California VA Hospital in Sacramento. Having a mobile computer is essential in order to continue working despite having two separate offices. This computer is also valuable in presentations and note taking a various meetings, reviewing data, finding funding opportunities, etc.
- **Set up an Intranet with South Africa**: We did not do this. The Research effort at UC. Davis already has established lines of communications and data collection. The research group was opposed to having a separate system involving South Africa. Instead all data sent from South Africa goes through the established protocol at UC Davis. Further communications using e-mail and Fax have proven to be more that adequate.
- **Take a course in Research Methodology**: The course offered in Research methodology started well before I was hired at UC Davis. As a nongraduate student I would be required to pay full tuition for the course.

- **Begin cataloguing personal data base of BPH specimens:** When I left private practice in Santa Cruz I carried with me the operative records of over 80 patients with BPH. Of interest to the grant are those who only had median lobe enlargement of the prostate, and had the median lobe resected. Over the period of the 3 months I was able to contact the pathology department at Dominican Hospital and Santa Cruz as well as the Santa Cruz Medical Clinic to receive permission to contact the patients. I contact the 21 patients whom I felt were evaluable most by phone but some by letter. We then were able to formulate a release of information from 18 of those patients. With these releases we were able to get the paraffin fixed archival blocks released to my lab, as well as pathology reports and clinical data on these patients. This was completed in early 1999.
- **Participate in twice weekly lab meetings at UC Davis:** The UC Davis Prostate Cancer Affinity group meets every Tuesday morning at 10:00. Senior investigators attend these meetings, Dr. deVere white, Dr Xien Jien Kung, Director of Research, graduate students, post docs as well. It is at this 2-hour meeting that everyone reports on the progress of his or her work. Since I am relatively new, I began to report on my progress almost weekly. It is at these meetings that much of the direction of my lab and research is done. At this meeting a significant amount of scientific exchange occurs. My increased knowledge of the field of molecular genetics stems mostly from this type of experience of listening to the work, methods and progress of a variety of research efforts including UPA, Tyrosine Kinases, a new p53 loss of function assay. Topics ranging from RTPCR, western, southern and northern blots, DNA Microarray's, Laser scanning microdissection, tissue cultures, cell transfections etc, are all practiced and the particulars of these methods, the problems encountered and the ways in which to use them are all discussed on a weekly basis, as they relate to each investigators research efforts. I also have a weekly meeting with a the small team that I have

begun working with including the lab technician I hired, a volunteer from Travis Army base, Dr. Arline Dietch, an experienced and seasoned Ph.D., working in the same field of immunohistochemistry as I am doing on this grant.

- **Inquire about taking graduate course in molecular biology:** As above inquiries were made into graduate courses in molecular biology. No structured programs were available. We did inquire about a summer program in epidemiology in Canada, but no funding was available for this. I am going to the AACR meeting in September of 1999. This however is outside the funding period for this grant.
- **Work with Dr. Rocke(biostatistician) for statistical analysis:** We began collaboration with Dr. David Rocke in February. After several rounds of e-mail and a phone discussion, we set up a two hour meeting to discuss the design of my proposed research the numbers, data collection etc. During that meeting we discussed the upcoming project. We collaborated on writing a department of defense grant and he was responsible for writing the draft of the statistical portion of that grant. Subsequently we also applied for a VA merit grant and he has appointed a full time Assistant Professor to work with me on my projects. This person is now listed as the biostatistician on the Department of Defense Grant that was submitted in May of 1999.
- **Identify review course on molecular biology of the cell.**- I have chosen to attend the course given by the AACR on Molecular Aspects of Metastasis to be given on September of 1999

Phase 2 Information consolidation, Project and Personal development

- **Finish cataloguing personal data base**: Our personal data base now consists of the following
  55 paraffin fixed archival specimens of African Americans Prostate Cancers from collaborators at Duke University.
  18 African Americans paraffin fixed archival specimens from David Grant Medical Center in Fairfield California. These samples were received after establishing collaboration with Dr. Chris Chermansky, Urologist at David Grant who also has come aboard as a volunteer in my laboratory.
  8 African American paraffin fixed archival specimens of Patients I have operated on in 1999 at the VA hospital at Mather in Sacramento
  37 paraffin fixed archival specimens acquired from Dr. Reif in South Africa, from Blacks and whites with prostate cancers
  18 paraffin fixed archival specimens from the BPH specimens I brought from Santa Cruz, Dominican Hospital
  2 specimens of fresh frozen prostate cancers removed at prostatectomy from African Americans in 1999. These specimens were acquired after submitting an IRB for acquiring prostate tissue to the VA in Sacramento. The IRB proposal went through three separate committees at the VA before approval. We now have approval to use both fresh and paraffin fixed tissue in our studies, from the prostate cancer patients we treat.
- **Begin the process of evaluating graduate student to assist in studies, etc.:** In early 1999 we hired a graduate technician to assist with our studies. She worked in our lab and along with me learned several techniques including flow cytometry, immunohistochemistry and laser microdissection. She was invaluable in assisting with writing our DOD grant, and helping to work out the protocols of BCL-2, p53 and Rb. After working for 4 months however it was apparent her skills were not up to par, and we decided to not renew the at will contract we had established with her. She finished up her work in late June 1999. Her work will be taken over by another senior lab technician, and the funds slated for her have been transferred to that technician (Salvatore Tuscano). An extension was requested so that he could finish the work she was not able to do, and therefore although we are able to submit this final report we did requested an extension of funding for the project so that he can finish immunostaining the samples in the data base. We have subsequently applied for additional technical support in our New Investigator grant of 1999.
- **Second three way call between DR. Reif, Dr. deVere White and myself:** I have taken over primary communication with Dr. Reif in South Africa. Recently we sent him a copy of the DOD 99 grant we

submitted, listing him as a collaborator as well as communicating with some issue to correct some discrepancies in the clinical follow-up of the patients blocks he had sent us.

- **Consult Bay area cancer registry to inquire about the status of archival CaP specimens of African Americans in the Bay area**: We did consult with the Northern California Cancer Registry as well as the California Cancer registry about this subject. Although many of the Hospitals in the Bay area keep blocks for many years, the process of contacting these patients for their consent, as well as getting appropriate follow-up would appear to burdensome to take on as a means of collecting samples of African American patients. We suspect that collaboration across the State of California with the other University of California would be a more fruitful way to gain access to clinical and archival material. In association with the University of California Research Departments and Urology departments we held a conference call in January with the Chairman or their representatives of the five UC campuses and decided to collaborate on this issue. In February I wrote a Special Pilot grant to the American Cancer Society to approve funds to organize this effort. Although that grant was subsequently turned down, we were able to find funds to organize that effort. A meeting of 17 investigators from the UC campuses was convened at UC Davis, July 18 and 19. Dr. deVere White and I moderated that meeting. At that meeting the participants agreed to form a consortium of the campuses to look at prostate cancer in ethnically diverse populations, and to right a major grant to fund this effort. At that meeting I was able to establish collaborations with Dr. William Aronson, Asst Professor at UCLA who at the VA hospital has access to a number of African American archival specimens. It is anticipated that with the Duke collaboration, the David Grant Air Force Hospital collaboration and the new UCLA collaboration and my taking over as the Chief of Urology at the VA Hospital and having an active protocol accepted by the VA to acquire specimens we will be able to acquire sufficient African American Archival specimens to run any studies for immunohistochemistry.
- **Begin preliminary immunohistochemistry of BCL-2, p53 and RB:** Because we had established protocols at UCD it was not necessary to work out new ones. Instead of running IHC on the specimens piecemeal we decided to await arrival from Duke University and South Africa. The South African specimens arrived in March and the Duke specimens arrived in June. Our initial run at the specimens met with poor success. Staining quality that we had originally seen with the BPH specimens and previous UC Davis reports was not present. We worked through the protocol and identified several problems. The most difficult was the DAB that we used to stain the specimens brown, was not working at the same levels as previously. We found that we had used DAB from two different sources and identified the possibility of a bad lot of reagent. Over the course of 3 weeks starting in June we have identified the problem, run through the appropriate positive and negative controls and feel confident that we have the protocol working. As of 2 days ago we have begin staining an initial set of 22 specimens of Prostate Cancer 11 from South Africa, and 11 from David Grant Air Force Hospital. We will submit these specimens to Dr. Regina Gandour –Edwards, the IHC Core Lab director and Asst Professor of Pathology, for analysis. If the slides sent to her are evaluable we plan on running the 50 specimens from Duke University, as well as the remainder of the David Grant and South African specimens. We will be able to compare these to a like group of Caucasian patients that the lab has previously evaluated using the same protocol for BCL-2, p53 and RB. Once all the specimens are evaluated we should be able to go over the data with the statistician and prepare to publish the results.
- **Send abstract to Keystone conference on apoptosis deadline December 1998:** I did not accomplish this task. The deadline was only 6 weeks after I began work at UC Davis, and was prior to the receipt of funds to start the work. When we sent the grant in the summer of 1998 we anticipated an earlier starting date than was supplied to us by the DOD.
- **Begin to learn microdissection techniques:** Along with my graduate technician I have begun to learn the technique. The laboratory has however several technicians able to perform the technique and as such although I have learned the skill, it is probable that more expert collaborators within the lab, under my direction will be performing this technique. We have established collaboration and actually submitted in the DOD New Investigator grant, a portion using this technique. Dr. Xiu Boa Shi, has developed a partial loss of function assay for p53. Once we have identified p53 positive cancers in the African American ans South Africans by immunohistochemistry, we will turn these specimens over to Dr. Shi. Dr Shi will then use the

laser capture microdissection technique to dissect out p53 overexpressing areas in the tumor. The dissected specimens will then be submitted to his partial loss of function yeast assay to see if the p53 abnormalities represent full or partial loss of function and evaluate potential racial differences among the specimens.

- **Continue participation in lab meetings:** I continue to participate in the weekly Affinity group meetings as well as continue to hold our weekly lab meetings dealing specifically with my research team only.

Phase 3: Formulation of Research Questions for DOD PCRP FY 99

- **Consolidate information obtained during phase two:** We have used the information consolidated in phase two. We submitted a grant to the Department of Defense (New Investigator in May of 99), This grant uses the process we began with this grant and adds several new potential markers of racial differences in CaP, including immunostaining of p21, p27, BAX, RET Kinase. We will have to begin to work out the protocols for RET Kinase and BAX. Hopefully we will obtain the funding to do so. As alluded to above have we have established a collaboration with Dr. Shi to evaluate p53 loss of function in tumors overexpressing p53.
- **Continue collaborative efforts in South Africa**- done, we talk to South Africa monthly. The next communication will go over the results obtained from Dr. Gandour Edwards gives us the result of the BCL-2, p53 and RB analysis of the 37 South African patients we now have in our database.
- **Continue to establish data base of African American patients locally**: Since I have taken over as the Chief of Urology at the VA Hospital in Sacramento, we have access through our protocols, all tissue of Prostate cancers removed in African Americans as well as a data base of 40 patients paraffin fixed archival biopsies of patients who have been irradiated at the Northern California VA system since 1993. We continue to collect specimens from David Grant Air Force base as they come in. We have also established contact with Dr. Aronson at the VA Hospital Northridge, as well as organized a consortium of the University of California campuses, that will develop a sharing arrangement for tissues in Ethnically diverse patients throughout the system. It is anticipated that this will allow us access to hundreds of specimens that we ordinarily would no have access to.
- **Attend Keystone conference and present data**: Although we did not attend this conference I was invited to participate in a conference at the National Cancer Institute in June 1999. This conference on Prostate cancer in African Americans allowed me to present some of the research on BCL-2 overexpression differences among races. Although this work was done before my arrival, the experience was invaluable. It allowed me to display my knowledge of the subject and answer questions. It also allowed me exposure to National investigators such as Gerald Coetzee, Otis Brawly, Gary Miller and Wael Sakr, all of whom do extensive research on Prostate cancer in African Americans., and all of whom presented their work and at the end of the two day session we had formulated a plan for future directions for research in this area at the National Cancer Institute and across the country.
- **Attend American Urologic Scientific Assembly**; I attended this five day meeting
- **Continue to gain knowledge and experience in the use of other techniques to study cellular behavior through participation at UC Davis Cancer Center laboratories**; The time I spend at the lab is very valuable. As described the lab is populated by a variety of senior investigators and my immediate supervisor is now Dr. Hsing-Jien Kung. The environment of the lab meetings has allowed me the exposure to help to decide my ultimate direction of my research as stated in the grant submitted to the DOD 99.

Key research accomplishments and reportable outcomes

- Go back into academic medicine
- Establish a laboratory
- Hire a staff
- Collaborate with senior investigators at UC Davis
- Write 3 Grants, DOD FY99 New Investigator(pending), Special Pilot Grant American Cancer Society (Not funded) and VA Merit Grant II (pending)
- Presentation at National Cancer Institute conference on Prostate Cancer in African Americans demonstrating knowledge and competence in the subject of molecular biology of prostate cancer, and establishing a national presence in this field.
- Named Collaborator on UC Davis Grant submitted to the Department of Defense Spring 1999, The Prostate Cancer Initiation Award, looking at Tyrosine Kinases as CAP Progression Markers and Therapeutic Targets Co-organizer of the University of California Consortium on Prostate Cancer in Ethnically Diverse populations. This group is in the process of applying for 2.5 million dollars in funds to establish a Prostate Cancer Cohort in California in association with the California Cancer Registry. I will have the primary responsibility of overseeing the writing of this grant in association with a team of epidemiologists, molecular biologists, clinicians and including Dr. Rocke, and researchers from the Five University of California campuses.

Conclusions

Unlike many of the grants I have applied for, this unique funding opportunity was to help me to establish an academic presence and create an environment where I can prepare to further my research and specifically write a follow-up grant, which I have accomplished. Over the course of the grant I have met the majority of the tasks listed. I have established a presence in the field I am interested in and presented at a meeting of the majority of investigators interested in the same subject. I have established a variety of collaborations that should assist me in pursuing this line of scientific endeavor. I have learned the subject by being present at weekly meetings and writing 3 separate grant proposals on the subject to help fund my efforts. We have established a database of patients, and set in motion a program and protocols to continue to collect data on an ongoing basis. The grant did not request that we complete any studies during the granting period, however we are actively pursuing that line of study. Although we have decided to submit a final report based on the work promised, we have asked for an extension of the funding so that we can finish doing the actual testing on the samples we have collected from places as diverse as South Africa, and Duke University.

So what: We have positioned ourselves to investigate the problem we proposed when we submitted this grant proposal. The problem is identifying whether there are molecular differences in Prostate Cancer that underlie the increased morbidity and mortality seen in African Americans compared to Whites, and whether those differences will be reflected in changes of tumor suppressor genes as Africans have immigrated to America.. We have collected the specimens, and begun to evaluate the data. We have established collaborations so that we can look at the problem from a variety of directions including a prospective cohort to evaluate this problem.

Personnel receiving support from research effort

Dr. David Rocke	BIOSTATISTICIAN
Dr. Simon Reif	CONSULTANT UROLOGIST IN SOUTH AFRICA
Eileen Yoshida	SRA-1 LAB TECHNICIAN
Salvatore Toscano	SRA-2 LAB TECHNICIAN